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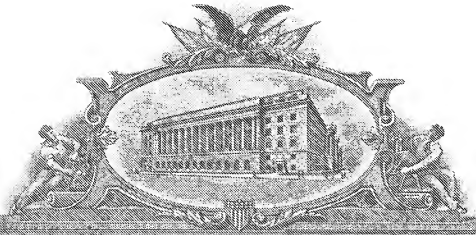

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This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

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INVENTOR(S)

Given Name (first and middle [if any])	Family Name or Surname	Residence (City and either State or Foreign Country)
Nan-Yao	Su	Davie, Florida

Additional inventors are being named on the _____ *separately numbered sheets attached hereto*

TITLE OF THE INVENTION (500 characters max)

Use of Ecdysteroids and Analogs Thereof for Controlling Termites

Direct all correspondence to: **CORRESPONDENCE ADDRESS**

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ENCLOSED APPLICATION PARTS *(check all that apply)*

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Respectfully submitted,

SIGNATURE _____

TYPED or PRINTED NAME Jay M. Sanders

Date February 19, 2004

REGISTRATION NO. 39,355 *(if appropriate)*

Docket Number: UF-412P

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Docket No. : UF-412P

Applicant(s) : Nan-Yao Su

For : Use of Ecdysteroids and Analogs Thereof for Controlling Termites

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USE OF ECDYSTEROIDS AND ANALOGS THEREOF FOR CONTROLLING TERMITES

Background of the Invention

[0001] Subterranean termites most often enter structures from the surrounding soil to feed on wood, or other cellulosic material, of the structure and its contents. If unchecked, termites can cause considerable damage. As a result, efforts to erect physical or chemical barriers to prevent the entrance of termites into a structure or to exterminate the termites after they have invaded a structure have proven a considerable expense to the public (Su, N.Y., J.H. Scheffrahn [1990] *Sociobiol.* 17(1):77-94). The cost to control termites in the United States exceeds 2.2 billion dollars annually (Su, N.-Y. [2002] *Sociobiol.* 40(1):95-101).

[0002] Subterranean termites construct an extensive foraging gallery beneath the soil surface. A single colony may contain several million termites with foraging territory extending up to 300 feet (Su, N.Y., R.H. Scheffrahn [1988] *Sociobiol.* 14(2):353-359). Since subterranean termites are a cryptic creature, their presence is not normally known until after some damage, foraging tubes, or live termites such as swarmers, are found. Some subterranean termites are known to forage beneath an object on the soil surface (Ettershank, G., J.A. Ettershank, W.G. Whitford [1980] *Environ. Entomol.* 9:645-648).

[0003] Currently, there are two basic approaches for the control of subterranean termites: preventive control and remedial control. In some of the United States, it is mandatory that the soil underlying the foundation of newly constructed buildings be pre-treated with a pesticide (also referred to herein as termiticide) to prevent termite infestation. Pesticide is typically sprayed over and into the soil prior to construction. This pre-construction treatment produces a horizontal barrier beneath the building. Because of the lack of communication between pesticide applicator and construction workers, the barrier often loses its continuity during the construction. Moreover, the currently available soil termiticides tend to lose their biological activity after five or more years to the extent that the treated soil is no longer effective against termite invasion. Established termite colonies in the soil may then invade the structure if additional chemical is not applied beneath and around the structure.

[0004] When a house or other building is infested by subterranean termites, one option is to create a continuous barrier beneath the building in the soil where the subterranean termites are provided access

to the building. A common method of creating this barrier is to introduce termiticide around a building foundation by injection into soil underlying concrete foundations, drenching the soil surrounding the building perimeter, or a combination of both. This type of post-construction treatment is labor-intensive and may not adequately produce a continuous barrier (Frishman, A.M., B.L. Bret [1991] *Pest Control* 59(8):48, 52, 54, 56; Frishman, A.M., A. St. Cyr [1988] *Pest Control Technology* 16(4):33, 34, 36).

[0005] Other remedial treatments include spot treatments such as dusting or injecting termiticides within the walls of the building. Robert Verkerk has described arsenic trioxide dust treatment using termite lures (Verkerk, R. [1990] *Building Out Termites*, Pluto Press Australia Limited, P.O. Box 199, Leichhardt, NSW 2040). Verkerk describes the use of stakes or blocks of termite susceptible timber to lure termites after the stakes or blocks have been placed near a known termite problem. Once termite activity is observed, arsenic trioxide is injected. Alternatively, a portion of the termites may be dusted with arsenic trioxide.

[0006] Some toxicants that have less environmental effect and that show activity against termites are known (Su, N.Y., M. Tamashiro, M. Haverty [1987] *J. Econ. Entomol.* 80:1-4; Su, N.Y., R.H. Scheffrahn [1988] *Florida Entomologist* 71(1):73-78; Su, N.Y., R.H. Scheffrahn [1989] *J. Econ. Entomol.* 82(4):1125-1129; Su, N.Y., R.H. Scheffrahn [1990] *Sociobiol.* 17(2):313-328; Su, N.Y. [1991] *Sociobiol.* 19(1):211-220; Su, N.Y., R.H. Scheffrahn [1991] *J. Econ. Entomol.* 84(1):170-175; Jones, S. [1984] *J. Econ. Entomol.* 77:1086-1091; Paton, R., L.R. Miller [1980] "Control of *Mastotermes darwiniensis* Froggatt (Isoptera: Mastotermitidae) with Mirex Baits," *Australian Forest Research* 10:249-258; McHenry, W.E., U.S. Patent No. 4,626,528; Henrick, C.A., U.S. Patent No. 5,151,443). However, these toxicants were not used in conjunction with a method that efficiently and efficaciously delivered the toxicant to a target pest.

[0007] The introduction of the first commercial termite bait system, SENTRICON®, in 1995 drastically changed the landscape of subterranean termite control practices. Traditional soil insecticide applications in the last half-century typically use 100-200 gallons (or 5-10 kg active ingredient) of pesticide to kill soil-borne subterranean termites in the immediate vicinity of the treated house. Because a subterranean termite colony may contain a gallery system that extends up to 300 feet from an infested house, soil treatments (despite the quantity of insecticide applied)

seldom impact the entire colony. A monitoring-baiting program such as SENTRICON®, on the other hand, is capable of eliminating the entire colony. *See* WO 93/23998, U.S. Patent No. 6,370,812, and U.S. Patent No. 6,397,516.

[0008] This type of system relies on periodic, routine monitoring. Stations containing monitoring devices are first placed in the soil to detect termites. When termites are found in a station, the monitoring device is replaced with baits containing a small amount of insecticide such as hexaflumuron. Termites are allowed to continue feeding on baits, which distributes the active ingredient throughout the colony population until the entire colony is eliminated. Because of the target-specific approach, only a few grams of hexaflumuron are needed to eliminate a colony that may contain several hundred thousand termites (Su 1994, *J. Econ. Entomol.* 87:389-397).

[0009] Due to its low environmental impact, hexaflumuron was the first compound to be registered under EPA's Reduced Risk Pesticide Initiative. The SENTRICON® system was the recipient of EPA's Presidential Green Chemistry Award in 2000.

[0010] There remains a need for additional termite toxicants that have little or no adverse environmental effects. For example, U.S. Patent Nos. 5,753,249; 6,214,364; and 5,558,862 relate to methods of controlling insects by administering enzymes that disrupt ecdysteroid metabolic pathways.

[0011] Insects have an exoskeleton (made of mostly chitin and proteins) that protects them from external elements such as weather and natural enemies. The external cuticle, however, has to be shed periodically for continuous growth. Endocrine products, most notably brain hormone, juvenile hormone, and ecdysone, are known to regulate insect molting (Chapman 1976). A general model for insect molting began with the secretion of brain hormone (or PTTH, prothoracicotropic hormone) from neurosecretory cells. The brain hormone is stored in the corpus cardiacum (a pair of organs below the insect brain and associated with the aorta) before being delivered to the prothoracic gland to stimulate it to produce ecdysone (Fig. 1A). Ecdysone is rapidly converted to 20-hydroxyecdysone (or "20E," Fig. 1B) following its release into the insect hemolymph (Nation 2002). These ecdysteroids activate a specific gene to produce protein in epidermal cells. Because insects are incapable of synthesizing steroids, ecdysone is believed to be obtained indirectly from the insect diet. Juvenile hormone (JH), a sesquiterpenoid, is produced by corpora allata (a pair of glandular bodies

situated at both sides of the esophagus) to regulate the type of protein being synthesized. The presence of JH ensures that insects retain the juvenile form (*i.e.* to molt from a younger larval stage to the next larval stage). As insects progressively molt, JH concentration decreases and may even be totally absent at the last larval instar. In the absence of JH, a larva then molts into pupa or adult stage.

[0012] The effects of JH, its analogs (JHAs), and mimicries (JHMs) on termites are well studied (Su and Scheffrahn 1990). JHAs and JHMs (referred to as juvenoids) are known to produce excessive soldier termites whose function is for colony defense. Because the soldier caste has to be fed by workers, termite colonies contain optimal proportions of the soldier caste, again, which apparently evolved to most efficiently provide for the defense of the colony (Wilson 1971, Haverty 1977). It has been proposed that juvenoids, which induce excessive soldier formation, may be used to disrupt the integrity of a termite society, leading to the destruction of the entire colony (Haverty 1977, Hrdy and Krecek1972, Hrdy 1973). Further studies revealed, however, that juvenoids are effective only against termite species with a lower natural soldier proportion (such as *Reticulitermes* species), but not those with higher soldier proportion such as *Coptotermes* species (Su and Scheffrahn 1990). Because *Coptotermes* species include an unusually large proportion of economically important termites in the world (Su 2003), and because juvenoids are ineffective against such an important genera of termite pests, juvenoids are not commercially used for termite control.

[0013] One relatively recent development in subterranean termite control is the use of termite baits containing chitin synthesis inhibitors (CSIs) such as hexaflumuron or noviflumuron to eliminate the vast colony of subterranean termites (Su 1994, Su 2003), which is an accomplishment that could not be achieved by use of traditional liquid insecticides. To eliminate a colony of 100,0000 – 1,000,000 termites that may forage up to 300 feet, the active ingredient (AI) for a bait has to be non-repellent, slow-acting, and its lethal time has to be dose-independent so that the AI is distributed throughout the colony by termites before the onset of death (Su and Scheffrahn 1998). Insect growth regulators (IGRs) such as juvenoids and CSIs satisfy all these three requirements. However, because many IGRs are species-specific, thus far only hexaflumuron and noviflumuron are successfully used to control multiple species of termite pests (Su 2003). CSIs generally inhibit the biosynthesis of chitin, but its complete process remains poorly understood (Nation 2002).

[0014] While juvenoids and CSIs are well investigated for their potentials for termite control, limited information is available for even the normal function of ecdysone in termites. Lüscher and Karson (1958) and Lüscher (1960), while trying to determine the role, if any, that ecdysone plays in the normal biology of termites (and not in attempts to control termites), reported that injection of ecdysone alone or in combination with JH induced a normal pseudogate molting of the lower termites, *Kalotermes flavicollis*. Since their studies, there has been no known investigation of the effects of ecdysone in or on termites.

[0015] Akin to juvenoids, synthetic versions of ecdysteroids have been used to control some insect pests of agricultural importance, but not termites. These analogs typically mimic the activity of 20E to cause premature molting (Wing *et al.* 1988). Toward the end of the molting under normal condition, 20E is degraded and excreted, thus allowing the eclosion hormone to complete the process (Nation 2002). The analogs, however, are more stable than 20E and are not easily degraded or excreted (Wing *et al.* 1998). Consequently, their continuing presence in insect hemolymph interferes with complete molting and causes hyperecdysonism (premature molting without a successful termination). Hence, these analogs can be referred to as ecdysteroid agonists (Dhadialla *et al.* 1998).

[0016] U.S. Patent Nos. 6,123,756 and 6,248,159 relate to wood preservative for protecting wood against dry-wood-destroying insects, such as the house longhorn (*Hylotrupes bajulus*), woodworm (*Anobium punctatum*), and bark beetle (*Lyctus brunneus*). Those patents relate to lumber (not insect bait) treated with a combination of a juvenile hormone and an ecdysone agonist. Subterranean termites are different from drywood termites, are not dry-wood-destroying insects, and are not mentioned or suggested in those patents. *See, e.g.*, U.S. Patent No. 5,027,546, which describes a system intended for use on above ground termites, *i.e.* drywood termites, by freezing them with liquid nitrogen.

[0017] Positive results were obtained when the ecdysteroid agonist RH-5849 was tested against some insect species (Darvas *et al.* 1992), but little if any information is available regarding potential effects of ecdysteroid agonists against termites. Raina *et al.* (2003) reported that one such agonist, halofenozide, may impact the reproductive physiology of alate nymphs of *C. formosanus*. However, for use in baits to eliminate a subterranean termite colony, the active ingredient in the bait has to be lethal to the worker caste, which makes up the majority of the colony population. Alate nymphs,

which make up only a small portion of a colony, eventually leave the nest to start a new colony somewhere else. However, they do not forage like workers. Thus, eliminating the young, alate nymphs would not impact the overall colony population and its damaging potential.

[0018] Although there have been some reports limited to testing of halofenozide against termites, there have been no reports of this causing any meaningful mortality against termites. *See e.g.* Monteagudo and Su (2002) and (2003), conference abstract and titles, relating to halofenozide testing on workers of the eastern subterranean termite (*Reticulitermes flavipes*) or the Formosan subterranean termite (*C. formosanus*). There was also a USDA inhouse memo that mentioned RH-0345 (a.k.a. halofenozide) as having a significant effect on ovarian development in termite alates and nymphs. However, the effect, at low doses used in the test, was temporary. That memo also discussed the use of a juvenile hormone analog to induce the formation of mutant soldiers. An abstract from the Florida Entomological Society 2002 Annual Meeting (Monteagudo & Su [2002]), related to preliminary results of a choice test conducted to examine preference, deterrence, and lethality of the insect growth regulator bait halofenozide on Eastern subterranean termites. A "ten-minute paper" (Monteagudo & Su [2002]) from the 2002 ESA Annual Meeting and Exhibition stated that halofenozide was evaluated in a choice test for its potential as a bait toxicant against the eastern subterranean termite, *R. flavipes*. Feeding blocks composed of wood slices were vacuum-impregnated with halofenozide at various concentrations, with feeding deterrence occurring at concentrations greater than 4,000 ppm. In summary, in these few reports, halofenozide showed little or no meaningful activity against termites.

[0019] U.S. Patent No. 6,093,415 relates to synergistic effects between juvenoids and CSIs in termite baits. Ecdysone and analogs thereof are not mentioned. The use and investigation of juvenile hormones might be another reason that ecdysone and analogs thereof were not heretofore identified as successful termite control agents.

Brief Summary of the Invention

[0020] The subject invention relates in part to the oral administration of ecdysteroids and analogs thereof to foraging subterranean worker termites. Preferred ecdysteroids for use according to the subject invention are ecdysone, certain analogs thereof, and 20-hydroxyecdysone, for example. After

eating ecdysteroids of the subject invention, termites were induced to molting. However, they could not complete the molting and eventually died. This is the first time that fatal effects of ecdysteroids against termites were demonstrated.

Brief Description of the Figures

[0021] **Figure 1A** shows the chemical structure of ecdysone. **Figure 1B** shows its active hormone 20-hydroxyecdysone.

[0022] **Figure 2** shows the chemical structure of tebufenozide, an ecdysone analog.

Detailed Description of the Invention

[0023] The subject invention relates in part to the oral administration of ecdysteroids and analogs thereof for controlling subterranean termites. When exposed to ecdyson and 20E-ecdyson (the by-product of ecdyson usually created inside insects), termites were induced to molting. However, they could not complete the molting and eventually died. This is the first known report of fatal effects of ecdysteroids against termites. Ecdysone and 20-hydroxyecdysone (20E) are preferred ecdysteroids for use in baits according to the subject invention. However, other ecdysteroids, in addition to ecdysone and 20E, can fed (or administered in food) to termites according to the subject invention, as explained in more detail below.

[0024] It was quite surprising to find that ecdysone and other analogs thereof provided excellent termite control. The subject invention provides exciting new options for inhibiting termites (*i.e.*, killing them and preferably the entire colony, making them sick, preventing them from feeding on wooden structures, and the like).

[0025] Compounds for use according to the subject invention can be used in the bait matrix of SENTRICON-like station housings, aboveground stations, and hermetically sealed baits. The subject compounds can be fed to foraging subterranean termite workers (and delivered by them to nestmates of the same colony). As an initial matter, unless specifically stated, when "termites" are referred to herein generically, such reference is to subterranean termites. Subterranean termites are different from drywood termites.

[0026] One relatively recent development in subterranean termite control is the use of termite baits containing chitin synthesis inhibitors (CSIs) such as hexaflumuron or noviflumuron to eliminate the vast colony of subterranean termites (Su 1994, Su 2003), which is an accomplishment that could not be achieved by use of traditional liquid insecticides. To eliminate a colony of 100,0000 – 1,000,000 termites that may forage up to 300 feet, the active ingredient (AI) for a bait has to be non-repellent, slow-acting, and its lethal time has to be dose-independent so that the AI is distributed throughout the colony by termites before the onset of death (Su and Scheffrahn 1998). Insect growth regulators (IGRs) such as juvenoids and CSIs satisfy all these three requirements. However, because many IGRs are species-specific, thus far only hexaflumuron and noviflumuron are successfully used to control multiple species of termite pests (Su 2003). CSIs generally inhibit the biosynthesis of chitin, but its complete process remains poorly understood (Nation 2002).

[0027] Although the slow-acting characteristic for CSIs against termites is desirable for the complete distribution of the AI into the vast colony for its elimination, the lengthy time required for the hexaflumuron baits to kill a colony could become its shortcoming on some occasions. The time period for such termite baits to eliminate a colony may be divided into three segments: 1) time required for the discovery of bait stations by termites, 2) time required for termites to ingest lethal dose, and 3) time required for termites to begin molting (thereby allowing the ingested CSIs to interfere with successful molting, leading to the death of the individual). The relatively more recent use of noviflumuron has shortened the 2nd segment (the time required to ingest a lethal dose). Noviflumuron is more lethal than hexaflumuron, and a smaller amount (and thus the time needed for ingesting lethal does) is required to reach the lethal dose. Because the effects of CSIs do not take place until molting process begins, the 3rd time segment remains the same when CSIs are used.

[0028] In this invention, the hyperecdysonism of ecdysone and 20E against *R. flavipes* and *C. formosanus* is described for the first time. These two termite species represent the most economically important genera of termites in the world. According to the subject invention, ecdysteroids and non-halofenozide-type ecdysteroid agonists can be used in bait matrices to cause delayed lethality of termite workers, leading to the elimination of a colony. Use of ecdysteroids or these ecdysteroid agonists has an advantage over CSIs because ecdysteroids actively induce molting instead of passively waiting for termites to molt before the effects can take place. Thus, the subject

invention advantageously (and surprisingly) removes the 3rd segment of time required for termite colony elimination. Ecdysteroids or the subject ecdysteroid agonists can be also used synergistically in combination with CSIs in baits so that termites that are exposed to a sublethal dose are induced to molt, yet the molting process is inhibited by the CSI.

[0029] It was quite surprising to find that ecdysone and other analogs thereof provided excellent termite control. When exposed to ecdyson and 20E-ecdyson (the by-product of ecdyson usually created inside insects; also called "20E"), termites were induced to molting. However, they could not complete the molting and eventually died. There might also be other adverse (but desirable) effects on the target termites, such as cessation of feeding and overt toxicity in affected termites. In addition, an effective amount of the ecdysteroid can simply be used to induce molting; this is helpful when used to "synergize" or enhance the effects of a CSI, as discussed above.

[0030] This is the first known report of fatal effects of ecdysone against termites. This discovery was completely unexpected and surprising, especially noting prior studies where injection of ecdysone into termites simply caused successful molting without any harmful effects. (Lüscher and Karson 1958; Lüscher 1960.) Ecdysone and 20E, and similar analogs, are preferred for use according to the subject invention. However, other ecdysone agonists, in addition to 20E but distinct from halofenozide, can be used according to the subject invention, as explained in more detail below. Halofenozide, also called RH-0345, is a coleopteran-specific variant of tebufenozide. Halofenozide showed little or no meaningful activity against termites. Thus, it is excluded from the subject invention.

[0031] Examples of ecdysteroid analogs for use, or adaptable for use, according to the subject invention include tebufenozide, which is an insecticide originally designed for controlling lepidopteran pests. It does not affect natural populations of beneficial, predatory, and parasitic insects for the control of other insect pests. **Figure 2** shows the chemical structure of tebufenozide, from the 1996 *Farm Chemicals Handbook*. RH-5849 (1,2-dibenzoyl-1-tert-butylhydrazine) is a beetle-specific alternative to tebufenozide. Some other ecdysteroid agonists include methoxyfenozide and chromafenozide. Thus, the subject invention can include the use of tebufenozide, tebufenozide analogues, RH-5849, and chromafenozide, but not halofenozide. Another possibility is methoxyfenozide or RH-2485.

[0032] Certain ecdysone analogs, which are growth regulators, may be more effective against certain species of termites. Many insect growth regulators (IGRs) can also be species-specific. Thus, they can be selected and optimized for a given situation. Prior to the disclosure of the subject invention, ecdysteroids and analogs thereof, other than halofenozide, were not investigated for their ability to control termites. However, in light of the disclosure of the subject invention, one will now consider ecdysteroids, such as ecdysone, analogs thereof, and byproducts thereof for use in termite control programs. In contrast to the state of the art prior to the subject invention, the fact that the natural hormones ecdysone and 20E showed positive results will now lead those skilled in the art to believe and expect that other ecdysone agonists, especially those having desirable structural characteristics, can now be used to advantageously control termites. In preferred embodiments, one can use ecdysone and 20E in baits to induce hyperecdysonism in foraging worker caste (and non-foraging by trophallaxis) subterranean termites.

[0033] With the foregoing considered, examples of termite species that can be targeted (selectively) by use of the subject methods include *Coptotermes formosanus*, *Reticulitermes flavipes*, *R. hesperus*, *R. virginicus*, *R. tibialis*, and *Heterotermes aureus*, as well as termite species of the families (and pest genera) Mastotermitidae (*Mastotermes* species), Hodotermididae (*Anacanthotermes*, *Zootermopsis* species), Rhinotermitidae (*Coptotermes*, *Heterotermes*, *Reticulitermes*, *Psammotermes*, *Prorhinotermes*, *Schedorhinotermes* species), Kalotermitidae (*Glyptotermes*, *Neotermes*, *Cryptotermes*, *Incisitermes*, *Kalotermes*, *Marginitermes* species), Serritermitidae, and Termitidae (*Pericapritermes*, *Allodontermes*, *Microtermes*, *Odontotermes*, *Nasutitermes*, *Termes*, *Amitermes*, *Globitermes*, *Microcerotermes* species), Termopsidae (*Hodotermopsis*, *Zootermopsis* species), and other pest species of termites. Preferably, methods of the subject invention are used to target subterranean termites.

[0034] Although the subject invention can be practiced in many ways, certain apparatuses are preferred for use according to the subject invention. Preferred apparatuses are described in WO 93/23998, U.S. Patent No. 6,370,812, and U.S. Patent No. 6,397,516.

[0035] Preferred apparatuses useful according to the subject invention comprise a housing that is designed to enclose a monitoring device and/or toxicant-containing matrix. This housing is useful for protecting the monitoring device and/or toxicant-containing matrix from the environment. The monitoring device or matrix can be enclosed within the housing in such a manner so they can be

removed with minimal disruption to the foraging termites. This housing is preferably made from a durable, non-biodegradable material. Preferably, once infested by termites, the monitoring device can be gently removed from the soil or from the station housing (it is advantageous to utilize a station housing to minimize disruption to foraging tunnels). Upon removal of the monitoring device, a toxicant-containing matrix comprising an ecdysteroid or an analog thereof can then be placed in the station housing. The monitoring device and the toxicant matrix preferably comprise cellulose.

[0036] Various other materials can optionally be used to encase the toxicant-containing matrix. This method for packaging the toxicant-containing matrix has the advantage of creating a "dose-pack" that precisely provides the appropriate amount of toxicant. "An effective amount" of the subject ecdysteroid toxicant can be administered that is sufficient to kill, make sick, and/or prevent termite feeding of the structure or area being protected. "An effective amount" also distinguishes over naturally occurring (relatively very low) levels of the ecdysone or analog thereof that might be found in nature. An "effective amount" for termite control can also distinguish over natural or non-orally administered amounts of ecdysone that cause the normal termite molting process.

[0037] In preferred embodiments, the ecdysteroid of the subject invention is administered/made available to foraging worker termites (is present in the toxicant matrix) at a concentration of less than 10,000 ppm, preferably at or below 7,500 ppm, more preferably at or below 5,000 ppm, and still more preferably at or below 1,000 ppm. Also preferred are concentrations of less that 4,000 ppm.

[0038] As mentioned above, the subject ecdysteroid or analog "AI" (active ingredient) can be used in conjunction with another toxicant or AI. The subject ecdysone-type AIs and other preferred AIs are slow-acting, lethal at concentrations which do not repel target insects, and capable of being combined with the matrix as described above. It is intended that pests directly contacting or ingesting the toxicant will not be killed immediately but will travel to and/or through their colony to recruit other nestmates to the toxicant, thereby resulting in the control of large numbers of colony members. It is preferred that the pest die days, weeks, or even months after encountering the toxicant of the subject invention.

[0039] The second active ingredient (the first AI being an ecdysteroid, or analog, of the subject invention) can preferably comprise chemicals that interfere with the formation of exo-cuticle such as the chitin synthesis inhibitors (CSIs). CSIs are known to interfere with the chitin synthesis procedure, but such procedure does not take place until insects produce ecdysone under a pre-determined, natural

biological clock. Thus CSIs have to passively wait for the natural molting to take place. When synergistically used in combination with CSIs, ecdysteroids or ecdysteroid agonists can induce termites to molt (after oral ingestion) even where the termites ingest a sublethal dose (a dose that may not cause hyerpecdysonism but will initiate molting), and then the molting process is inhibited by a CSI. Examples of preferred second AIs, as mentioned above, are hexaflumuron, noviflumuron, diflubenzuron, azadirachtin, lufenuron, and other acryl ureas. Specific examples of preferred second AIs, as mentioned above, are hexaflumuron and noviflumoron, which can be impregnated or incorporated into cellulose material, preferably, during the formulation of the toxicant-containing matrix.

[0040] Again, there are a variety of methods and apparatuses that can be utilized to practice the subject invention. The precise methods and apparatuses can be selected for optimal control of a particular target pest and environmental setting. Such applications would be apparent to a person skilled in this art using the teachings provided herein. For example, for particularly "shy" species of termites, a toxicant of the subject invention can be selected accordingly and used in the hermetically sealed baits described in WO 03/082000 and U.S.S.N. 10/392,730. Bait stations using the subject toxicants can also be made more "attractive" to termites (generally and/or specifically) by using the pheromones and semiochemicals and non-cellulose polymer delivery devices of WO 03/092376 and U.S.S.N. 10/392,798.

[0041] In light of the surprising new properties of ecdysteroids reported herein for the first time, other aspects of the subject invention include methods of screening an ecdysteroid, an ecdysteroid analog, and ecdysone analogs and byproducts (but not halofenozide) for inhibitory activity against a type of subterranean termite. Methods of such screening are known in the art and some are exemplified herein. However, as discussed herein, there was no motivation, heretofore, to conduct such screens.

[0042] All patents, patent applications, provisional applications, and publications referred to or cited herein are incorporated by reference in their entirety to the extent they are not inconsistent with the explicit teachings of this specification.

[0043] Following are examples that illustrate procedures for practicing the invention. These examples should not be construed as limiting. All percentages are by weight and all solvent mixture proportions are by volume unless otherwise noted.

Example 1 – Protocol for feeding ecdysone to subterranean termites and determining the effects thereof

[0044] Termites were collected from three colonies each of *C. formosanus* and *R. flavipes* and were held in the laboratory at 26 ± 1°C and 98 ± 2% RH before use. Technical grade ecdysone was dissolved in methanol to obtain 0.1, 1, 10, 100 and 1,000-ppm solutions by serial dilution. Methanol solutions (*i.e.*, 0 ppm AI) were used as untreated controls. Each concentration solution (0.2 ml) was pipetted onto 55-mm-diameter Whatman No. 1 filter paper fitted into 5-cm-diameter glass Petri dishes and allowed to evaporate overnight.

[0045] This filter paper served as the cellulose food source for twenty-five termites, plus one soldier for *R. flavipes* or three soldiers for *C. formosanus*, which were introduced into each Petri dish after moistening the filter paper with 0.175 ml deionized water. For each species, two subsamples each of three colonies were used per concentration for a total of 72 experimental units. The bioassay units was held at 26 ± 1°C. Observation was made daily for 12 days. Termites showing symptoms of incomplete molting were counted, and dead termites were removed from each unit. Because affected termites did not recover, they were included in mortality data. For each treatment and species combination, mean percent termites showing symptoms of incomplete molting and mortality among concentrations at 12 day were arcsine-root transformed and subjected to the analysis of variance (ANOVA). Significant differences ($\alpha = 0.05$) among concentrations were separated using Fisher's least significant difference (LSD) test (SAS Institute 1999).

Example 2 – Results of feeding ecdysone to subterranean termites

[0046] Symptoms of hyperecdysonism (incomplete molting) were evident for both termite species after exposure to ecdysone at 1,000 ppm (Tables 1). After approximately 7 days of exposure, some termites exhibited the "jackknife" position due to the incomplete molting. The symptoms were similar to those exposed to the chitin synthesis inhibitor, hexaflumuron, as reported by Su &

Scheffrahn (1993), or those observed from larval Coleopterans and Lepidoptera when treated with ecdysone agonists, where death occurs after premature molts are initiated but not terminated (Dhadialla *et al.* 1998). This is the only second time such incomplete molting symptom was recorded from termites (the first time being those caused by CSIs). With CSIs, termite molting was inhibited after ecdysone initiated the natural molting under a pre-set biological schedule. With the ecdysone exposure as described in this experiment, termites were artificially induced to premature molting without a successful termination. This is the reason why the symptoms of incomplete molting appeared faster in ecdysone (7 days) than in CSIs (4-8 weeks). Significant mortalities at 12 day were recorded for both termite species exposed to ecdysone at >100 ppm (Table 1), with a large proportion of *R. flavipes* showing symptoms of hyperecdysonism. Surprisingly, 100% mortality was recorded from *C. formosanus* exposed to 100-ppm ecdyson. *C. formosanus* is generally less responsive to IGRs than *R. flavipes*, but with ecdyson, this appears to be the reverse.

Table 1. Percent termites exhibiting incomplete molting (± SE) and mortality (± SE) of *R. flavipes* and *C. formosanus* after 12-day exposure to ecdysone.

Concentration	% Incomplete Molting		% Mortality	
(ppm)	*R. flavipes*	*C. formosanus*	*R. flavipes*	*C. formosanus*
0	0.00 ± 0.00a	0.00 ± 0.00a	0.64 ± 0.64a	0.60 ± 0.60a
0.1	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	1.79 ± 1.22a
1	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	1.19 ± 0.75a
10	0.00 ± 0.00a	2.38 ± 2.38a	0.00 ± 0.00a	11.31 ± 6.55a
100	0.00 ± 0.00a	8.33 ± 5.95a	60.26 ± 18.06b	100.00 ± 0.00b
1,000	21.80 ± 14.10b	3.57 ± 2.26a	100.00 ± 0.00c	93.45 ± 6.55b

Means within a column followed by the same letter are not significantly different ($\alpha = 0.05$; ANOVA [SAS Institute 1999]).

Example 3 – Protocol for feeding 20-hydroxyecdysone to subterranean termites and determining the effects thereof

[0047] 20-hydroxyecdysone is an ecdysone byproduct. Technical grade 20-hydroxyecdysone (20E) was dissolved and used in the same manners as was ecdyonse, as discussed above in Example 1. The results are presented in the following example.

Example 4 – Results of feeding 20-hydroxyecdysone to subterranean termites

[0048] Symptoms of hyperecdysonism were evident for both termite species after exposure to 20E at 1,000 ppm (Table 2). After approximately 7 days of exposure, some termites exhibited the "jackknife" position due to the incomplete molting, as discussed above in Example 2. As with ecdysone, 20E also caused incomplete molting at a faster rate than CSIs.

[0049] Significant mortality was recorded from *R. flavipes* exposed to >100 ppm 20E, with 100% mortality for those exposed to 1,000 ppm (Table 2). *C. formosanus* was less responsive to 20E than *R. flavipes*, showing ca. 75% mortality at 1,000 ppm. Nonetheless, results of ecdysone and 20E were much superior than that for halofenozide, which caused only 50% mortality for *C. formosanus* at 10,000 ppm when tested under similar condition (Monteagudo and Su 2002).

Table 2. Percent termites exhibiting incomplete molting (± SE) and mortality (± SE) of *R. flavipes* and *C. formosanus* after 12-day exposure to 20-hydroxyecdysone.

Concentration	% Incomplete Molting		% Mortality	
(ppm)	*R. flavipes*	*C. formosanus*	*R. flavipes*	*C. formosanus*
0	0.00 ± 0.00a	0.00 ± 0.00a	1.28 ± 0.81a	0.60 ± 0.60a
0.1	0.00 ± 0.00a	0.00 ± 0.00a	0.64 ± 0.64a	1.19 ± 0.75ab
1	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	10.12 ± 9.42bc
10	0.00 ± 0.00a	0.00 ± 0.00a	0.64 ± 0.64a	3.57 ± 2.26abc
100	3.85 ± 1.40ab	2.38 ± 1.19a	76.19 ± 11.28b	11.90 ± 5.43c
1,000	7.69 ± 3.29b	26.19 ± 6.09b	100.00 ± 0.00c	74.40 ± 3.62d

Means within a column followed by the same letter are not significantly different (α = 0.05; ANOVA [SAS Institute 1999]).

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Abstract

[0050] The subject invention relates in part to the oral administration of ecdysteroids for controlling subterranean termites. Preferred ecdysteroids for use according to the subject invention are ecdysone, certain ecdysone analogs, and 20-hydroxyecdysone, for example. Preferably, one or more compounds of the subject invention are used in a termite bait alone or in combination with one or more chitin synthesis inhibitors to cause delayed mortality of termites to control their populations.

Fig. 1A

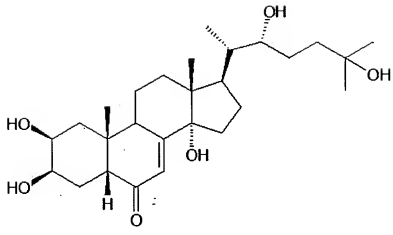
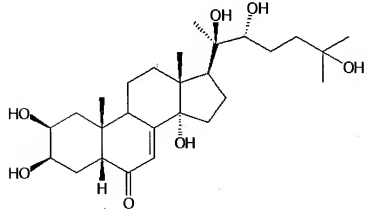
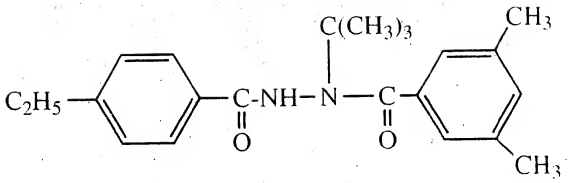


Fig. 1B





Tebufenozide

Fig. 2